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Original Article

Preliminary Survey of Larval Trematodes in Freshwater Snails of Phitsanulok Province in Lower Northern Thailand

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Abstract

Background: Freshwater snails serve as intermediate hosts for a variety of trematodes that cause illness in the human and animal populations. Several species of freshwater snails in Thailand have been found to have larval trematode infections. We aimed to investigate a freshwater snail in Phitsanulok Province and report on its current status of larval trematode infection.

Methods: Freshwater snails were collected from six localities (rice field and irrigation canal) by handpicking and using a count per unit of time sampling approach. The snails were identified by their external shell morphology. The shedding method was applied to observe the cercariae, which were photographed under a light microscope to determine their morphological types.

Results: A total of 211 snails were classified into seven genera. The most abundant snail species was *Lymnaea* sp., representing 31.3% of the sample, followed by *Physella* sp., *Bithynia* sp., *Pomacea canaliculata*, *Filopaludina martensi*, *Indoplanorbis exustus*, and *Melanoides tuberculata*, in that order. From the sample, 21 snails (9.95%), including *Bithynia* sp., *Lymnaea* sp., *I. exustus*, and *M. tuberculata*, were infected with cercarial trematodes, which could be categorized into four types, namely amphistome, parapleurolophocercous, echinostome, and xiphidiocercaria. Amphistome emerged from *Bithynia* sp., and *I. exustus* was the most common cercaria to be recovered, representing 80.9% of all infected snails.

Conclusion: This study presents the current prevalence of cercariae in infected snails within the studied area. It is important to manage intermediate host snails in order to restrict trematode life cycle completion.

Introduction

Trematode infections in humans and animals are a major helminthic disease and are major agents of morbidity and mortality worldwide, particularly in underdeveloped countries (1). Digenetic trematodes have a complicated life cycle, which required snails to be the first or the second intermediate host (2). When snails act as the first intermediate host, the larval stages of trematodes follow the sequence of sporocysts, rediae, and cercariae. The cercarial trematodes are released from the infected snail host to find a new host for their transmissions (3, 4).

Several species of freshwater snails become naturally infected by larval trematodes (5–9), especially in the north and northeastern part of Thailand where their prevalence is high (10–12). For example, *Melanoides tuberculata* was found to be infected with parapleurolophocercous, pleurolophocercous, and megalurous cercariae. Moreover, *Bithynia siamensis siamensis* was found to be infected with monostome, gymnocephalus, and virgulate cercariae. Moreover, snails in the Planorbidae family act as intermediate hosts of animal schistosomes in the family Schistosomatidae, which is the cause of cercarial dermatitis in humans (13, 14). Trematode infections have been reported among intermediate hosts in Thailand (15, 16).

However, only a few studies on snail diversity and cercarial infection have been reported in Phitsanulok Province, which is in the lower northern part of Thailand. This province is an important agricultural area with a large number of rice fields, which serve as a habitat for a variety of intermediate host snails. It is situated on the geographical line uniting the central and northern regions of Thailand. Phitsanulok consists of 9 districts where the mountain region which is one third covers the east and north part of province. We randomly selected 3 districts to collect the snail samples; namely Mueang Phitsanulok District, Bang Rakam, and Wang Thong District. These areas are the plain region with most rice paddy fields and irrigation canals.

Hence, the objectives of this study were to examine a freshwater snail from Phitsanulok province, Thailand, and report on its current larval trematode infection status. The results of this investigation will provide a foundation for preventing or controlling intermediate host snails, which may aid in limiting trematode infection in the study area.

Materials and Methods

Snail collection

The research was carried out in 4-28 February 2019. For the study, six sites in Thailand's Phitsanulok province were surveyed for freshwater snails. A total of three rice fields in Mueang Phitsanulok District (RM1, RM2, and RM3), two rice fields in Bang Rakam District (RB5 and RB6), and one irrigation canal in Wang Thong District (CW4) shows in Fig. 1 and Table 1.

Collection and identification of freshwater snails

Snail specimens were collected by handpicking or using a wire-mesh scoop with a count per unit of time sampling method (17). Two researchers collected snail samples for 30 minutes at each site. All snails collected were cleaned, labelled, and stored in plastic bags. The snails were returned to the laboratory to be identified for genus or species based on external shell morphology using taxonomic keys (18). Following that, the snails were examined for trematode larvae (cercariae).

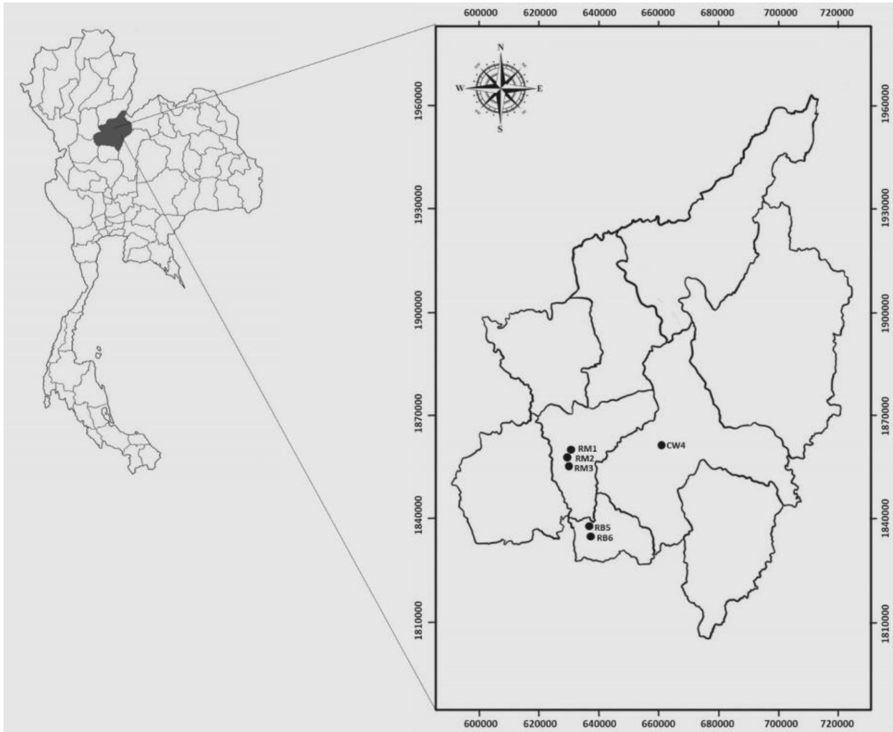

Fig. 1: Map of the Phitsanulok province showing areas from where freshwater snails were collected

Table 1: Species and the number of freshwater snails collected from six localities in the Phitsanulok province

Site of collection (code)	***Number of snails***							***Total***
	Bithynia sp.	*M. tuberculata*	*P. canaliculata*	*F. matensi*	*Lymnaea* sp.	*Physella* sp.	*I. exustus*	
Rice field, Mueang Phitsanulok district (RM1)	20	-	6	14	1	-	1	42
Rice field, Mueang Phitsanulok district (RM2)	-	-	1	1	25	-	-	27
Rice field, Mueang Phitsanulok district (RM3)	-	1	4	1	40	-	-	46
Irrigation canal, Wang Thong District (CW4)	-	-	1	3	-	-	1	5
Rice field, Bang Rakam District (RB5)	-	-	-	-	-	30	-	30
Rice field, Bang Rakam District (RB6)	15	-	19	-	-	26	1	61
Total	35	1	31	19	66	56	3	211

Examination for larval trematode infections

Using cercarial shedding procedures, the snails were investigated for trematode infections. Each snail was washed and placed in a plastic cup with 20 ml of dechlorinated tap water. Cercarial shedding was carried out for 24 hours at room temperature under natural light (19–21). Under the stereomicroscope, trematode cercariae were studied and their swimming activity was noted. The cercariae were collected using a plastic pasture pipette from unstained and mounted on slides with a heat fix. A camera was used to record images of immobile cercariae beneath a compound microscope. According to Schell, Yamaguti, and Ito, morphological types of cercariae were classified based on the observed characteristics (22–24).

Results

In the current investigation, a freshwater snail was collected in February 2019 from six distinct locations in Phitsanulok Province of Thailand. The collected snails were divided into seven families, seven genera, and seven species (Table 1 and 2). *Lymnaea* sp. was the most prevalent species, accounting for 31.3% of the sample, followed by *Physella* sp., *Bithynia* sp., *P. canaliculata, F. matensi, I. exustus*, and *M. tuberculata* (Fig. 2). *Pomacea canaliculata* was discovered in five locations, but *M. tuberculata* was discovered once in a rice field in Mueang Phitsanulok District (RM3). Snail diversity is abundant in rice fields, particularly in RM1, where five different kinds of snails were discovered.

Cercariae infection was found in four species of snails, including *Bithynia* sp., *Lymnaea* sp., *I. exustus,* and *M. tuberculata* (21 out 211 snails or 9.95%) (Table 2). The observed cercariae were then classified into four categories (Fig. 3 and Table 3): amphistome, echinostome, parapleurolophocercous, and xiphidiocercaria. After echinostome cercaria, amphistome that emerged from *Bithynia* sp. and *I. exustus* was the most prevalent cercaria, accounting for 80.9% of all infected snails (9.5%). In each cercarial type, xiphidiocercaria and parapleurolophocercous cercaria constituted 4.7% of all infected snails. *Bithynia* sp. were reported to be infected with amphistome cercaria and xiphidiocercaria in two separate locations (RM1 and RB5, respectively).

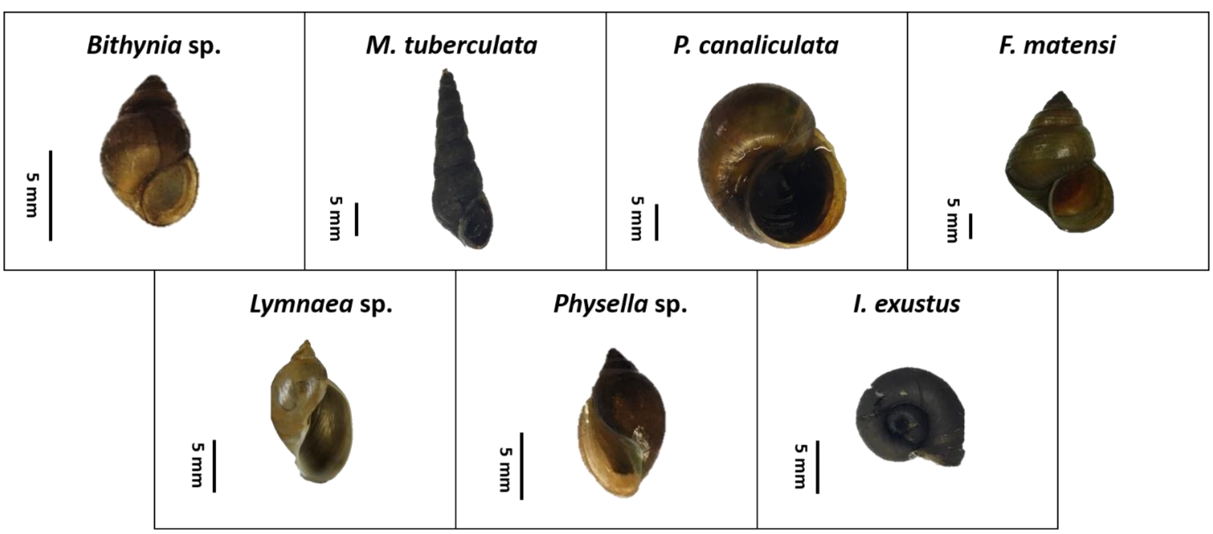

Fig. 2: The freshwater snails collected in this study

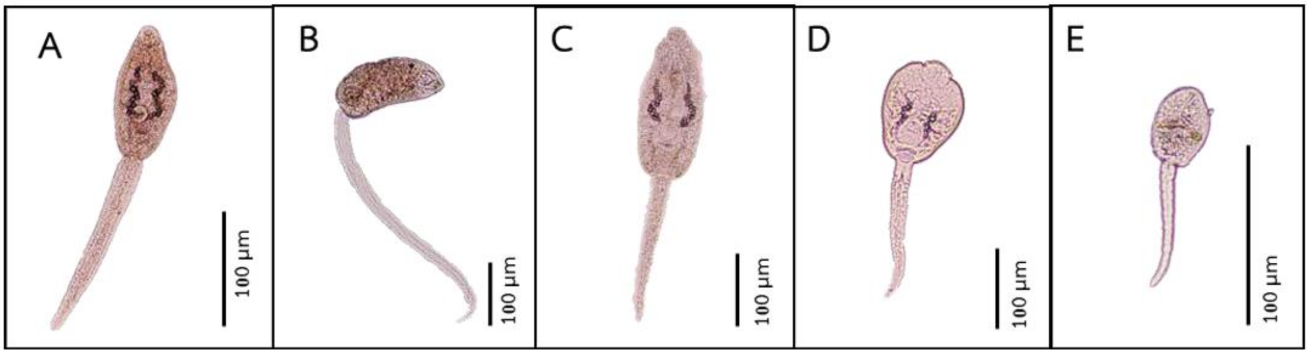

Fig. 3: The cercariae emerged from freshwater snails. (A) amphistome cercaria from *Bithynia* sp., (B) parapleurolophocercous cercaria, (C) amphistome cercaria from *I. exustus*, (D) echinostome cercaria, and (E) xiphidiocercaria

Table 2: Number of infected snails with larval trematodes

Snails collected in the present study			***No. of snail collected***	***No. of infected snail***	***Number of infected snail from each site***					
Family	Genus	Species			RM1	RM2	RM3	CW4	RB5	RB6
Bithyniidae	*Bithynia*	*Bithynia* sp.	35	17	1	-	-	-	-	16
Physidae	*Physella*	*Physella* sp.	56	2	-	-	-	-	2	-
Planorbidae	*Indoplanorbis*	*I. exustus*	3	1	-	-	-	-	-	1
Thiaridae	*Melanoides*	*M. tuberculata*	1	1	-	-	1	-	-	-
Viviparidae	*Filopaludina*	*F. matensi*	19	0	-	-	-	-	-	-
Ampullariidae	*Pomacea*	*P. canaliculata*	31	0	-	-	-	-	-	-
Lymnaeidae	*Lymnaea*	*Lymnaea* sp.	66	0	-	-	-	-	-	-
Total			211	21	1	-	1	-	2	17

Table 3: Cercariae types in the infected snails

Cercariae types	***No. of infected snail***	***Infected snail species***
Amphistome cercaria	16	*Bithynia* sp.
Xiphidiocercaria	1	*Bithynia* sp.
Echinostome cercaria	2	*Physella* sp.
Amphistome cercaria	1	*I. exustus*
Parapleurolophocercous cercaria	1	*M. tuberculata*

Discussion

Our research determines the current prevalence of trematode infection in a freshwater snail in Phitsanulok province of Thailand. A total of 9.95% (21 out of 211) infected snails, with a species distribution of *Bithynia* sp., *Lymnaea* sp., *I. exustus,* and *M. tuberculata*, were discovered. The prevalence rate (9.95%) of freshwater snails with trematode infection in the current study is comparable to that determined by a survey conducted in Chiang Mai (9.6% or 19 out 352 samples) (25). The rate is higher than several previous studies, for example, 1.38% (17 out of 1,227 samples) in Chiang Rai province (8), 6.20% in five provinces of Northern Thailand (24), and 1.69% (35 out of 2,076 samples) in Ubon Ratchathani Province (26). In Chiang Mai Province, however, the infection rate of snails with trematodes was 17.27% (11). The prevalence of cercariae infections in freshwater snails is well-reported globally, such as 16% in Sri Lanka (27), 4.3% in Nepal (28), 27.9% in Iran (29), and 6.6% in Zimbabwe (30). When compared to earlier studies, the infection rate reported in the current study is rather high. This may involve different factors, including differences in geographical localities of water reservoirs, season, snail species in each water area, and cercarial type (31–32).

In general, snail populations in different geographical areas fluctuate depending on the amount of rainfall, since their habitats are usually swept away by heavy rains. The surviving snails take a few months to resettle and reproduce in order to increase their populations (31-32). The snail specimens collected for this study were collected in the month of February, which is a few months away from the rainy season in Thailand. Seven species of snails were found in the studied area, namely *Lymnaea* sp., *Physella* sp., *Bithynia* sp., *P. canaliculata, F. martensi, I. exustus* and *M. tuberculata*. The most abundant species was *Lymnaea* sp., representing 31.3% of the entire sample, in contrast to other provinces where *Bithynia sp.* was found to be the most abundant (8, 11). Snail populations were higher in rice fields, with five snail species being found in one locality. Similar to a previous study, snail diversity was higher in rice fields than in other habitats (8). The distribution pattern of freshwater snails was rather erratic as a result of differences in water habitat, anthropocene alteration of water environments, and agricultural activities such as transplanting or harvesting and using chemicals, some of which may contain molluscicide (33).

Cercarial emergence was seen in four different species: *Bithynia* sp., *Lymnaea* sp., *I. exustus,* and *M. tuberculata*. In this investigation, the remaining three species, *P. canaliculate, F. martensi,* and *Physella* sp., were not infected by any type of cercariae. Amphistome cercariae were found in the greatest abundance in *Bithynia* sp. and *I. exustus*. In Nepal, they were discovered in *I. exustus, Gabbia orcula,* and *Gyraulus euphraticus* (28). This cercaria is responsible for snail-borne illnesses, amphistomiasis in domestic animals, and on rare occasions, amphistomiasis in humans (34–35). Xiphidiocercaria was observed in *Bithynia* sp., correlating with previous reports that this form of cercariae was found in three snail species, namely, *B. siamensis, M. tuberculata,* and *T. granifera* (11). Furthermore, *B. siamensis goniomphalos* could act as an intermediate host for seven different species of cercariae (25). This shows that *Bithynia* sp. snails are trematode intermediate hosts. Moreover, *Lymnaea caillaudi, Cleopatra bulinoides, Lonistes carnatus, Thiara tuberculata*, and *Thiara granifera* were hosted by xiphidiocercaria (36–37).

As shown in the present study, parapleurolophocercous cercariae were found in *M. tuberculata*. Earlier, parapleurolophocercous was observed in thiarid snails, including *M. tuberculata, M. jugicostis, Thiara scabra, T. granifera,* and *Sermyla riquetii* (8, 11, 38). The morphological characteristic of parapleurolophocercous cercaria was identified as belonging to the family Heterophyidae, which includes *Haplorchis taichui, Haplorchis pumilio, Stellantchasmus falcatus, Centrocestus caninus*, and *Procerovum* sp. (39–44). Echinostome cercariae were discovered in physellid snails, whereas several studies reported its emergence in viviparid, planorbid, lymnaeid, and thiarid snails (8, 45–47), which were reported to be the cercarial stage of the intestinal trematodes in the family Echinostomatidae (48).

The four larval trematodes found in the present study showed the ability to infect several snail species. Similarly, snails collected for this study were shown to be intermediate hosts that are susceptible to harboring a wide spectrum of cercarial types. As a result, freshwater snails can be considered an important determinant for monitoring the health of human and animal populations.

Conclusion

We report the prevalence of larval trematodes in freshwater snails in the Phitsanulok Province of Thailand. Four types of larval trematodes with health-related implications for humans and animals were found in freshwater snails in the studied area. These findings suggest that it is necessary to control the snail population in this area in order to check the prevalence of snail-borne diseases.

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Conflict of interest

The authors declare that there is no conflict of interest.

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